TITLE:

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CITATION:

Yamagata, Hirotada ...[et al]. Effects of Temperature on the Induction of Somatic Mutation by Acute Gamma Radiation Exposures in Rice Plants (Special Issue on Physical, Chemical and Biological Effects of Gamma Radiation, XI). Bulletin of the Institute for Chemical Research, Kyoto University 1970, 48(1): 72-77

ISSUE DATE:

1970-03-31

URL:

http://hdl.handle.net/2433/76321

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Bull. Inst. Chem. Res., Kyoto Univ., Vol. 48, No. 1, 1970

Effects of Temperature on the Induction of Somatic Mutation by Acute Gamma Radiation Exposures in Rice Plants

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Received January 30, 1970

Radiation damage and especially somatic mutation induced by gamma irradiation were studied paying special attention to the effects of temperature. Potted plants of rice variety Gimbōzu were exposed to 4~12 kr of gamma rays at various stages of plant development in 1968. Plants derived from Gimbōzu and heterozygous for a color gene were exposed to 4~20 kr of gamma rays at tillering stage in 1969. Exposures at every developmental stage were conducted at 10°, 20° and 30°C simultaneously. Radiation damages in several characters and somatic mutations were examined in both years and in 1969, respectively. Experimental results indicated remarkable effects of temperature on both the degree of damage and the frequency of mutation. Considerations were made on relationship between dose effect and temperature and on somatic mutation. The 2000-Curie cobalt-60 gamma-ray irradiation facility for biological studies was used for the study.

I. INTRODUCTION

The primary interest for plant improvement by means of induced mutation is how to obtain greater efficiency of mutation induction. The yield of gene mutation involves two components, *i. e.*, mutation rate and survival rate at each level of cell, tissue, organ and plant. The latter is closely related to the degree of physiological and chromosomal damages leading to undesired selection of mutation. These two components, unfortunately, behave contrary to each other after mutagenic treatment: higher radiation dosage increasing the former decreases inevitably the latter. Efficient obtaining of the mutation, therefore, may be realized on a fine balance between the two.

Numerous radiobiological studies have shown that, so far as sparsely ionizing radiations are concerned, various sorts of environmental factors involving temperature are responsible for modifying the effects of radiations. This seems to suggest the possibility of increasing mutations without decreasing survivals. In other words, the condition for efficient obtaining of gene mutation may be elucidated from analytical studies of the radiobiological effects of environmental factors.

On the other hand, early determination of mutagenic effects of radiations has been strikingly demanded in studies on mutation induction. In higher plants, however, the mutation rate has been estimated generally by scoring the frequency of mutants in the X_2 generation. An advantageous way to determine the mutagenic effects is to use plants heterozygous for a color allele and to score the somatic

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mutation sectors appearing on the X_1 plants. Since the first attempt[1] in 1956, radiation-induced somatic mutations have been studied in several plants, such as *Antirrhinum*,[2~4] *Lilium*[3,4] *Tradescantia*,[3,4] *Petunia*,[3,4] *Zea*,[5,6] *Impatiens*,[7] *Trifolium*,[8,9] *Avena*[10~13] and *Cosmos*.[14] However, few works have been conducted with an aim of detecting the effects of environmental factors.

From the view points above, growing rice plants normal and hetorozygous for a color gene were irradiated by gamma rays from cobalt-60 source, and the mutual relationships among dosage, factor modifying radiation effect, damage and mutation induced were studied. Estimation of the role of temperature on the mutagenic effect of gamma rays is the main subject of the present paper. The experiment was conducted using the 2000-Curie cobalt-60 gamma-ray irradiation facility for biological studies,[15] making the most of its characteristics.

II. MATERIALS AND METHODS

In 1968, a number of potted plants of rice variety Gimbōzu were prepared for experiments. 4, 8 and 12 kr of gamma rays from cobalt-60 source were administered to the plants at various developmental stages from seedling to anthesis. "Acute" (1557 r/h) and "chronic" (108 r/h) irradiations were applied for each stage group: the latter was started just after the former and covered 5 days with 4-hour pause every day. Exposure was made at three different temperatures, 10°, 20° and 30°C, independently but at the same time for the same stage group. Besides, non-irradiated control plants were kept under the same environmental conditions as those of the irradiated plants during irradiation, for checking the environmental effects. 12 plants were used for each treatment, and several characters were examined on radiation damages inclusive of seed sterility.

In 1969, a line segregating albina seedlings, induced by X-irradiation of Gimbōzu in 1961, was used as the material. It had been confirmed that this line only involved a recessive gene mutation responsible for white color, *i. e.*, being an isogenic line. The green plants, containing both normal and heterozygous ones, were potted and exposed to gamma rays at the tillering stage (21 July~2 Aug.). 4, 8, 12, 16 and 20 kr of exposure were given acutely (267~1000 r/h, 15~20 hrs.) under the same conditions as those in 1968. Each treatment comprised three replications I, II and III, which was treated within 21~23 July, 23~25 July and 31 July~2 Aug., respectively. Soon after heading time, several upper leaves emerged after treatment were observed (5~10 Sept.) for all the tillers of all plants used, and somatic mutation sectors shown as white stripes were carefully scored leaf after leaf. The heterozygosity of each plant was checked by progeny test with ripe seeds. Observed data on white sectors were arranged with heterozygous plants. Radiation damages inclusive of seed sterility were also examined.

III. RESULTS

1. Radiosensitivity

Yamagata *et al.*[16] suggested through X-irradiation of growing rice plants at several developmental stages that the occurrence and degree of damage caused on

X_1 plants varied with the developmental stage as well as with the character, and that the degree of damage in each character increases linearly with the dose of X rays in all stages. Results from the present experiment supported those suggestions, and further clarified remarkable effects of environmental temperature on radiation responses.

Of the results obtained in 1968, effects of temperature on the relationship between developmental stage and seed fertility are shown in Fig. 1. Exact identification of each developmental stage at the time of irradiation could not be made by reverse operation of days from heading date, because of growth disturbances due to the abnormal low temperature of late summer in 1968. However, it seems valid to infer from the rigid observation by Yamagata *et al.*[16] that the panicle primordiums began to differentiate at the beginning of August and reached meiosis on about the 23rd of August. So it can be said that the response of seed fertility to radiation is accelerated after the beginning of differentiation till meiosis. Here it is worthy of attention that temperature modifies the response markedly; the lower, the greater.

No considerable difference was observed between acute and chronic irradiations as Fig. 1 shows. As to the chronic irradiation, however, there were found wide fluctuation depending upon the stage at the time of exposure, and it is supposed that remarkable physiological damages were caused by lower temperature as seen with non-irradiated controls. Therefore, it appears difficult to discuss the effect of temperature on exposure rate or duration.

Figure 2 shows the response of seed fertility to exposure at tillering stage in 1969. It is observed in this figure that the dose effect is linear irrespective of temperature but remarkably increases with lowering of temperature. However, it is also observed that the degrees of dose response fairly differ from those obtained at the same stage and temperature in 1968 (Fig. 1). The reason why such

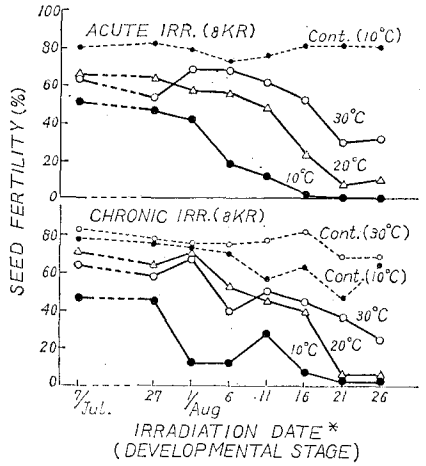

Fig. 1. Relationship between developmental stage and seed fertility (1968).
* Showing the first day of each chronic irradiation.

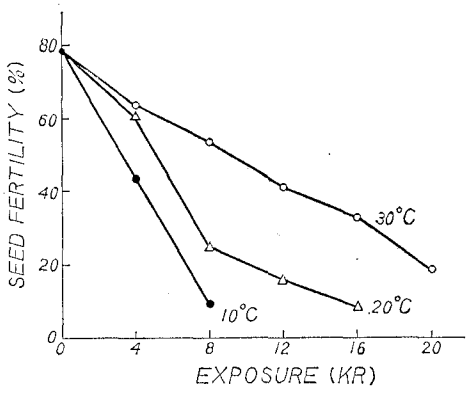

Fig. 2. Dose response of seed fertility (1969).

a difference appeared is not yet clear.

2. Somatic mutation

The progeny test proved that totaling 285 plants, composed of 239 irradiated and 46 non-irradiated plants, were heterozygous for the specific gene locus. 13,953 leaves from those heterozygous plants were examined on the somatic mutation sector. Of these leaves, 11,533 were from irradiated plants: the unmber in each treatment ranged from 549 to 1,255.

Various-sized white stripes were observed on 1,304 leaves of the irradiated plants, while no obvious aberration was found on any leaf of the control plants. Very narrow and short stripes, however, were excluded in case of arranging data, because they were undistinguishable from some small aberrations resulted from any causes other than radiation exposure. Consequently, clear stripes to be used for data were observed on 1,219 leaves.

The great majority of these leaves had only one stripe respectively, and no leaf carring four or more stripes was found out. In most cases, those stripes were observed on five upper leaves for two replications I and II, and four upper leaves for replication III. Besides, it was a general tendency that younger leaves had larger stripes. This fact agrees with the observation by Nishiyama *et al.*[11]

Somatic mutation frequency of each treatment was expressed by the number of stripes per leaf blade. The dose responses of the frequency are shown in Fig. 3, in which each frequency is exhibited together with the range of three replication values. Figure 3 shows that mutation frequency is strongly affected by temperature, namely, larger dose response is caused by lower temperature. Figure 3, however, also shows that the maximum frequency may be obtained rather at medium temperature (20°C). According to Fig. 3, each of the three different dose responses is not linear but increases with some power of the exposure and reaches respective satulation point.

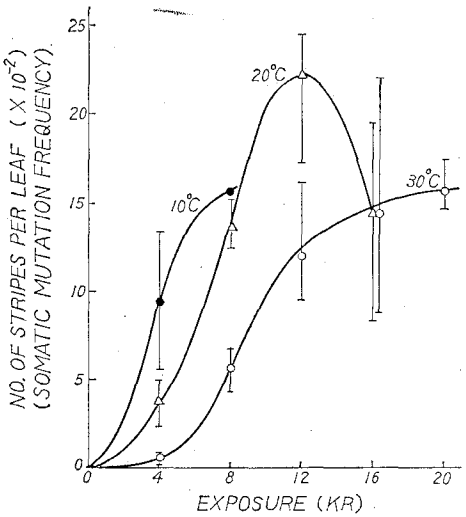

Fig. 3. Dose response of somatic mutation frequency (1969).

IV. DISCUSSION

Experimental results revealed that the temperature at the time of irradiation gave a remarkable influence on mutation frequency as well as radiation damage. Sparrow *et al.*[4] indicated through chronic irradiation of *Antirrhinum* that seasonal difference produced almost twice difference in mutation rate. They also showed[4] in *Tradescantia* that irradiation at 12.5°C brought about a 2.9-fold mutation rate compared with that at 21°C. Thus the dependency of mutation frequency upon temperature seems to suggest the possibility of efficient control of mutation by means of temperature.

The results obtained also indicated that decreased temperature increased both the degree of damage and the frequency of mutation. This fact may be due to dose accumulation per nucleus resulting from prolonged mitotic cycle time at decreased temperature. Leaving reasoning out of the question, however, the inevitable fact that temperature acts on both damage and mutation thus in the same direction is of course undesired for practical purpose. Accordingly, the most favorable balance of exposure and temperature should be sought for obtaining higher mutation yield. Taking the data of Figs. 2 and 3 into consideration, 12 kr and 20°C is considered to be in the most adequate balance so far as the present experiment is concerned. Combining such the favorable balance and any other modifying factors, *e. g.*, developmental stage, atmosphere such as oxygen and nitrogen, *etc.*, more advantageous conditions may be given for increasing mutation yield.

A few attentions should be paid to the utility of somatic mutation especially in seed-propagated plants. After all, the quick determination of mutagenic effects is the strongest point of the use of somatic mutation. It seems to be favorable also that fewer plant materials are needed for irradiation and higher dosage can be given, because X_2 seeds are required only for the determination of heterozygosity. On the contrary, it appears unfavorable that specific heterozygous materials must be prepared and not only heterozygous but also homozygous plants must be examined. Parallelism between induction of somatic mutations and that of mutations with practical characters may be taken as an essential problem for plant improvement.

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